

Lethbridge iGEM Collegiate 2014 Notebook – CELL CULTURE (August)

NOTE: For detailed protocols regarding starting/passaging/freezing/lipofecting cell cultures and preparing media, see “Cell Culture Protocols” document.

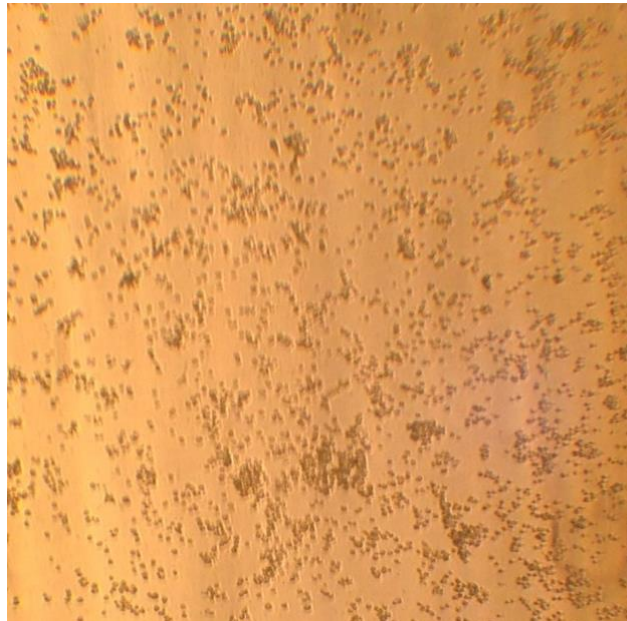
August 6, 2014

- Started HEK-293 cell culture plate and 8-chambered slide from 2 aliquots of frozen cell stocks.

August 7, 2014

- Changed media in HEK-293 cultures.

August 8, 2014



HEK-293 culture as seen under a light microscope.

- Lipofected HEK-293 slide culture with DNAfectamine (as per manufacturer's protocol):
 - 2 chambers: No transfection controls
 - 2 chambers: pcDNA3.0-Clover (1ug DNA and 10ul DNAfectamine per chamber)
 - 4 chambers: 2 each of 2 separate plasmid stocks (1ug DNA and 10ul DNAfectamine per chamber) of pcDNA3.0-Lamp2B-Clover



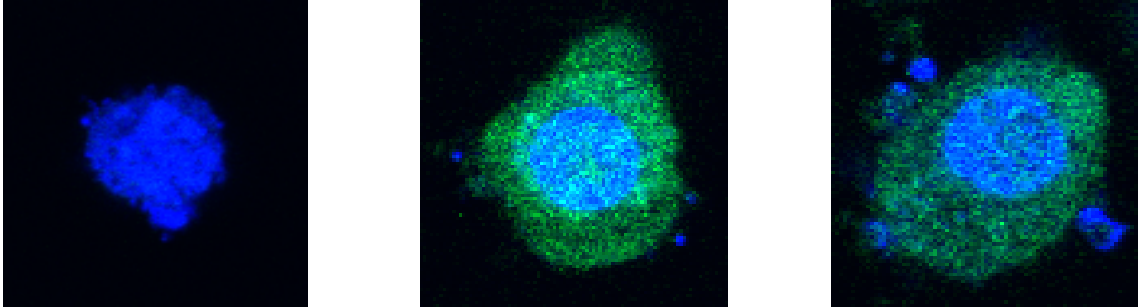
Chambered slide containing lipofected HEK-293 cell culture.

August 9, 2014

- Replaced serum-free media in lipofected HEK-293 culture with complete media.
- Changed media in non-lipofected HEK-293 plate culture.

August 11, 2014

- Changed HEK-293 media in plate culture.
- Tested fixation of cells: Aspirated media from lipofected HEK-293 slide culture and replaced one row with 4% paraformaldehyde (PFA) for 10 minutes at room temperature. Rinsed both rows with 1x phosphate buffered saline (PBS) and counterstained nuclei with 4',6'-diamidino-2-phenylindole (DAPI; 1:2000) for 5 minutes. Coverslipped with Vectashield and sealed slide with nailpolish.
- Fixation was not adequate – the vast majority of adherent cells were washed off in both unfixed and fixed rows. Will do a 20 minute 4% PFA fixation step in the future.
- Imaged slide on Olympus Fluoview FV1000 confocal laser scanning microscope. DAPI staining was also not adequate – will coverslip with Vectashield with DAPI in the future. Both Clover and Lamp2B-Clover lipofected cells demonstrated punctate cytosolic green fluorescence above control levels (likely attributable to appropriate Clover expression and folding).



Non-lipofected control HEK-293 cell nucleus (left) versus pcDNA3.0-Lamp2B-Clover lipofected HEK-293 nucleus (middle) and pcDNA3.0-Clover (right).

August 13, 2014

- Changed media in HEK-293 plate culture.

August 15, 2014

- Changed media in HEK-293 plate culture.
- Started one additional HEK-293 plate culture from a frozen cell stock.

August 16, 2014

- Changed media in HEK-293 plate cultures.

August 18, 2014

- Changed media in HEK-293 plate cultures.

August 20, 2014

- Changed media in HEK-293 plate cultures.

August 22, 2014

- Changed media in HEK-293 plate cultures.

August 25, 2014

- Changed media in HEK-293 plate cultures.

August 27, 2014

- HEK-293 cell cultures were not reaching confluence and were discarded.